SUPPLEMENTAL GENETICS MEMO

Sources and Sinks: Elucidating Mechanisms, Documenting Patterns, and Forecasting Impacts

SERDP Project RC-2120

JANUARY 2017

Samantha Hauser
Paul Leberg
University of Louisiana at Lafayette

Joshua Lawler
Julie Heinrichs
University of Washington, Seattle

Page Intentionally Left Blank

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
01/18/2017

2. REPORT TYPE
Supplemental Memo

3. DATES COVERED *(From - To)*
05/05/2011– 01/18/2017

4. TITLE AND SUBTITLE
Sources and Sinks: Elucidating Mechanisms, Documenting Patterns, and Forecasting Impacts

5a. CONTRACT NUMBER
W912HQ-11-C-0053

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Heinrichs, Julie, A.; Walker, Lauren, E.; Heimbuch, Michael, R.; Lawler, Joshua J.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

School of Environmental and
Forest Sciences, University
of Washington, Box 352100;
Seattle, WA 98195-2100

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program
4800 Mark Center Drive, Suite 17D03
Alexandria, VA 22350

10. SPONSOR/MONITOR'S ACRONYM(S)
SERDP

11. SPONSOR/MONITOR'S REPORT NUMBER(S) RC-2120

12. DISTRIBUTION / AVAILABILITY STATEMENT

Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A comprehensive understanding of source-sink dynamics can be critical for successfully managing at-risk species. We used field and modeling approaches to better understand the emergence and stability of sources and sinks and assess the implications of future system changes on population persistence. Modeling results identified the types of species and landscapes that are likely to exhibit strong source-sink dynamics and multi-species results indicated that management actions based on assumptions that sink habitats are generally harmful (or helpful) risk undermining conservation efforts. Black-capped vireo (*Vireo atricapilla*) field data and analyses documented source-sink patterns that were influenced by inter-annual variation and cowbird control on and near Fort Hood Military Installation in Texas. Most habitats outside of Fort Hood behaved like population sinks, and models indicated that persistence was sensitive to rates of inter-population exchange and the effects of future habitat restoration and climate change). Comparisons of black-capped and white-eyed vireos yielded insights into the unique limitations faced by black-capped vireos including a reduced breeding period and narrower use of habitat. This project highlights the importance of understanding source-sink dynamics in spatially-structured populations, as well as the need for applied source-sink theory and methods for conserving declining species in complex and changing landscapes.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:

17. LIMITATION OF ABSTRACT

18. NUMBER OF PAGES

19a. NAME OF RESPONSIBLE PERSON
Josh Lawler

Page Intentionally Left Blank

Note

This memo documents a supplemental genetics study that was funded as part of RC-2120. RC-2120 explored source sink dynamics using a combination of demographic field studies of the black-capped vireo (*Vireo atricapilla*) and theoretical and applied population modeling. The work described here was funded partway through the project and completion of the genetic work occurred after the final report was submitted. The genetic study was conducted by Dr. Paul Leberg and Samantha Hauser at the University of Louisiana at Lafayette.

Key Findings

- Of the 10 sampled sites, we identified 6 genetically distinct populations.
- Our results indicate on-going, but low level gene flow among these populations
- **Fort Hood is likely a source population for the region**
  - Fort Hood, particularly West Range Fort Hood, provides the majority of immigrants to other populations.
- Open rangeland and pasture likely provide the greatest barriers to movement, and conversely, connectivity is facilitated by scrub habitat

Background

Populations are often spatially structured. Understanding the effects of spatial structures such as sources and sinks, metapopulations, patchy networks, and isolated populations on population dynamics is critical for successful management. SERDP project RC-2120 explored source-sink population dynamics using a combination of demographic field studies of the Black-capped Vireo (*Vireo atricapilla*) and theoretical and applied population modeling. Field-based estimates of immigration and emigration for the study relied on resighting banded birds. However, even with over 600 banded birds and >300 natal dispersal records, we did not have enough resightings among different populations to estimate movement between regional habitat patches. With fewer than 10 records of natal dispersal events among on- and off-Fort Hood populations, and exchanges only among proximate populations, we could not quantify the degree of movement among other populations.

Population genetics provides an opportunity to estimate dispersal in Black-capped Vireos independent of band recoveries. Black-capped Vireos are small, less than 10 g in mass, making long term tracking via telemetry difficult due to the weight restrictions on protected migratory birds. Despite increased abundances, restricted gene flow has been documented in the species through the use of allozyme and microsatellite molecular markers (Fazio *et al.*, 2004; Barr *et al.*, 2008; Athrey *et al.*, 2012a). The Black-capped Vireo is a migratory bird, capable of flying to habitat patches well past the restricted movement inferred by the previous studies (Leberg *et al.*, 2009). However, despite their high vagility, male Black-capped Vireo philopatry to breeding territories may be driving the observed pattern of genetic differentiation (Athrey *et al.*, 2012a).

Fragmentation in central Texas has created habitat patches dependent on Black-capped Vireo dispersal. While some habitat patches are fairly large and contiguous like that on Fort Hood, others are much smaller such as those on private property. These patches likely have different growth rates producing source-sink dynamics around Fort Hood (Fazio *et al.*, 2004; Walker *et al.*, 2016). Walker *et al.* (2016) found demographic evidence for source-sink dynamics surrounding Fort Hood driven by Brown-headed Cowbird parasitism and habitat patch size. Additionally they concluded that the population dynamics in this system are complex and dynamic.

This supplemental study was designed to better understand the dynamics of movements between sites with breeding Black-capped Vireos. Our objectives were to 1) use genetic makers to elucidate gene flow around Fort Hood, which provides nesting habitat for the largest, most stable population(s) of Black-capped Vireos and 2) use resistance-surface modelling to better understand how landscape features influence gene flow and movement in and around Ft. Hood.

Objectives

1. **Estimate gene flow and dispersal for Black-capped Vireos between sites on and off Ft. Hood.**

2. **Assess potential landscape barriers to movement of Black-capped Vireos around Ft. Hood.**

Hypotheses

1. We hypothesized that the Ft. Hood populations of Black-capped Vireos would largely serve as source populations and thus we would expect that most of the documented movement would be from the base to the outlying populations.

2. We also hypothesized that open grassland, cropland, human development and water bodies would increase resistance to gene flow, whereas forest, elevation, and scrub would decrease resistance to gene flow.

Approach

Estimating gene flow and dispersal

Samples collection and DNA extraction and analysis

Blood samples from Black-capped Vireos were collected from 10 sites throughout Fort Hood [East Range (ER), East Fort Hood (EF), Manning Mountain (MM), Ridge Road (RR), West Fort Hood (WF) and West Range (WR)] and the surrounding central Texas habitat patches [Bessent/Byrd Property (BB), Balcones Canyonlands NWR (BC), Barnett Ranch (BR), Colorado Bend State Park (CB)] in central Texas in 2014 and 2015 (Figure 1). Black-capped Vireos were captured using a mistnet with Black-capped Vireo, White-eyed Vireo (*V. griseus*), or Eastern Screech Owl (*Megascops asio*) song playback. Each

bird was banded with a unique U.S. Geological Survey band and three color bands, with a unique color pattern. Toenail clips and pin feathers from Black-capped Vireos were immediately stored in Queen's Lysis Buffer at 4 ºC until DNA extraction.

We extracted genomic DNA from toenail clip and pinfeather samples using the Qiamp Micro DNA Kit Protocol for Isolation of Genomic DNA from Small Volumes of Blood. We genotyped samples at 12 species-specific microsatellite loci using the primers, *BCVI2-1*, *BCVI2-2*, *BCVI2-4*, *BCVI2-5*, *BCVI2-6*, *BCVI2-7*, *BCVI4-1*, *BCVI4-2*, *BCVI4-3*, *BCVI4-5*, *BCVI4-6*, *BCVI5-1*, PCR concentrations and cycling conditions from Barr et al. (2007), with the addition of 0.1 mg/ml BSA (bovine serum albumin) to each sample to increase PCR yield. Two loci (*BCVI 2-3* and *BCVI 4-4)* were excluded due to inconsistencies in amplification success. Each PCR product (1 ml) was added to 9.5 ml of Hi-Di Formamide (Applied Biosystems) and 0.5 ml of ROX 400HD size standard and run on an ABI 3130 Genetic Analyzer. Alleles at each locus were scored automatically using GENEMAPPER software and manually checked for error. All homozygotes and an equal number of heterozygotes were run three times to confirm their genotypes and to determine patterns of genotyping errors. In these, and in subsequent analyses, sequential Bonferroni corrections were used to adjust alpha levels to control Type I error rates in multiple, related comparisons (Rice, 1989).

Population Structure and Connectivity

We performed tests for deviations from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), and null alleles in GENEPOP 4.2 (Raymond and Rousset, 1995). We also used GENEPOP 4.2 to calculate observed heterozygosity, expected heterozygosity, and F_{IS} to evaluate genetic diversity in Black-capped Vireos at each study site. We used the hiefstat program in R to calculate allelic richness for each study site. To determine whether populations differed in genetic diversity, we performed a randomized block ANOVA on allelic richness and expected heterozygosity using study site as treatment and blocking by locus.

We used several approaches to elucidate population structure and gene flow among our study sites. Population differentiation was estimated with pairwise F_{ST} tests in GENEPOP 4.2. Population structure was also assessed using STRUCTURE and TESS. In STRUCTURE, we ran all 343 individuals at 12 loci with the admixture model to determine the number of clumps (k) present with our study sites. We evaluated k values from 1 to 7, with 10 iterations, 100,000 burn-in period and 100,000 MCMC (Monte Carlo Markov Chain) repetitions. The value of k with the lowest DIC value was chosen as the appropriate number of clumps for our system. We used location data as a prior. In TESS, we imputed 343 individuals at the 12 loci using an admixture model to also determine k. We evaluated k values from 2 to 7, with 10 iterations, 50,000 replications and 100,000 sweeps. The value of k with the lowest DIC value was chosen as the appropriate number of clumps for our system. Location data was used as a prior. STRUCTURE and TESS output was submitted to CLUMPAK to average membership coefficients over all runs for a given value of k. We used the R code provided by TESS to create kriging plots to visualize clustering.

We evaluated for the effect of isolation by distance (IBD), the positive relationship between linearized F_{ST} and Euclidean distance, at the population and individual level. At the population level, we tested for IBD using the R function IBD.test in the adegenet package and the mantel.test function in the PopGenReport package. At the individual level,

we tested IBD and spatial autocorrelation across all alleles in the program ALLELES IN SPACE according to the program's default settings. The spatial autocorrelation analysis in ALLELES IN SPACE calculates A_y which represents the genetic distance between two individuals over a given distance y. A_y values of 0 represent when all individuals are genetically identical and A_y values of 1 represent when all individuals are completely dissimilar. For all IBD evaluations, we used 343 individuals at 12 loci.

Migration

To further explore movement patterns among our study sites, we used BAYESASS, MIGRATE-N and GENECLASS2 to elucidate migration. We estimated migration rates in BAYESASS as the fraction of individuals from population i into population j. Parameters were kept with the default settings of the program. In MIGRATE-N, we estimated the number of migrants from population i to population j, including all permutations between populations. We implemented this procedure using a continuous Brownian motion data model for microsatellite markers as it approximates a discrete stepwise mutation model. Our parameterization was characterized by a constant mutation rate, a variable theta, and symmetrical migration rates. F_{ST} values were used for the prior distribution in a Bayesian inference search strategy. Other modeling options used the default settings of the program. We estimated gene flow more directly by detecting first generation migrants in GENECLASS2. We detected migrants using the Paetkau et al (2004) simulation algorithm and criterion and parameterized the simulations with a 0.01 allelic frequency, 0.01 p-value threshold and 1000 simulations. We estimated population numbers for our Fort Hood sites by multiplying the proportion of Black-capped Vireo habitat in each study site by the total number Black-capped Vireos estimated in Fort Hood (8000 individuals). Our central Texas site population estimates were derived from the number of territories found on our sites. These population estimates acted only as a comparative reference when considering numbers of migrants between sites and should not be used as true abundance data.

By assessing the populations of offspring-parent pairs, we can more directly look at dispersal between populations. We assigned individuals to candidate parents in CERVUS to more directly elucidate gene flow among our study sites. For individuals to be used in this assignment, we required a minimum of 6 loci genotyped. Simulations of parentage, both maternity and paternity, based on allele frequencies were performed to assess statistical significance. We considered any individual that was aged ASY (after-second-year) or older in the field to be a candidate parent, and all younger individuals to be candidate offspring. The most likely parent-offspring pairs were those with the highest LOD (likelihood of odds ratio). Both strict (95%) and relaxed (80%) confidence intervals were used when assessing the parentage assignments. We calculated the odds that the candidate parent came from the same population as that of the offspring (indicating residency versus migration). We calculated all odds using the odds ratio function in the R package fmsb.

Initial tests of allele frequencies differences indicated that some sites on Fort Hood were not genetically differentiated. Sites on Fort Hood that were not significantly differentiated and in close proximity were combined to produce three overall Fort Hood sites and 6 sites in total. This combining of nearby sites with genetically similar

composition was done because treating subsamples of a single population as different populations can bias downstream results. East Range combined (ER_c) included EF and ER individuals, and West Range combined (WR_c) included WR, MM, RR, and TA individuals. The remaining analysis used the resulting 6 sample sites: BB, BC, CB, ER_c, WF, and WR_c.

Elucidating landscape features that influence gene flow and movement

To assess the potential impact of landscape features on gene flow and movement, we used the genetic samples described above. We evaluated for the effect of isolation by distance (IBD), the positive relationship between linearized F_{ST} and Euclidean distance, at the population level. We tested at the population level as we are looking at interpopulation resistances. We tested for IBD using the R function IBD.test in the adegenet package and the mantel.test function in the PopGenReport package. We tested for spatial autocorrelation using the spautocor function in the R package PopGenReport.

We used GIS layers of land cover and elevation in our resistance modeling. Land cover was represented with separate rasters of forest (deciduous, evergreen, mixed and forested wetlands), scrub, open habitat (grassland and pasture), cropland, development, and water. Extents, projection, and coordinate systems, and cell sizes were identical among our raster files. To optimize the parameters of each landscape feature, we created 4 rasters with resistance surfaces based on our hypothesized response. Forest and scrub rasters were parameterized with values of one denoting any other land cover type, and 0.5, 0.1, 0.01, or 0.001 denoting the respective land cover type. Open habitat, cropland, development and water were parameterized with values of 1 denoting any other land cover type, and 2, 10, 100, or 1000 denoting the respective land cover. Elevation was parameterized using the raw continuous values. We created resistance surfaces for each of the resulting parameterized rasters using CIRCUITSCAPE with a node file of our 6 study sites.

To optimize the parameterization of our resistance surfaces, we ran a linear mixed effect model with only univariate model statements using the R package lme4. We used F_{ST}, as genetic distance, as the response variable, each parameterized landscape feature as the fixed effect, and population as the random effect. The lowest AICc score per landscape feature was chosen for the multivariate analysis. After checking for multicolinearity, we ran a linear mixed effect model as per above with our hypothesis model statements. Multivariate model statements include the full model, land cover, landscape features with hypothesized high resistance, landscape features with hypothesize low resistance, and landscape features with AICc values lower than distance. Univariate model statements include all landscape features. Euclidean distance was not added to any of our aforementioned model statements, as distance is implicit in the resistance matrices. Euclidean distance was also included as our null hypothesis.

Results

We sampled 343 individuals at our 10 study sites over the 2014 and 2015 summers (Table 1). There were no deviations from HWE after a sequential Bonferroni correction,

except at the Barnett Ranch (BR) site which may have been due to a small sample size (n = 5). We removed that sample from further analyses reducing our sample size to 338 individuals, except where noted otherwise. None of the locus pairs were out of LD for any population following a sequential Bonferroni correction.

Table 1. Summary of sample size, and average allelic richness (A_r), observed heterozygosity (Ho), and expected heterozygosity (H_e) over 12 loci. Standard errors are in parentheses. Letter indicate membership in the same non-significant subsets based on a Tukey test with a type I error rate of 0.05.

Sample Site	Sample Size	A_r	H_o	H_e
Central Texas				
BB	38	10.4 (0.1) AB	25.2 (1.1) B	29.1 (1.3) C
BC	27	8.9 (0.8) B	18.8 (0.9) C	20.1 (0.6) F
CB	20	10.0 (1.0) AB	14.4 (0.3) D	15.9 (0.2) G
Fort Hood				
ER	42	10.0 (1.0) AB	26.9 (1.9) B	32.2 (1.5) B
EF	34	10.8 (1.1) A	22.8 (1.2) BC	25.5 (1.0) DE
MM	32	9.3 (1.1) AB	22.9 (1.2) BC	25.6 (0.6) DE
RR	28	9.2 (1.0) B	18.8 (1.0) CD	21.1 (0.7) F
TA	35	9.8 (1.2) AB	24.4 (1.0) B	27.6 (1.0) CD
WF	32	10.0 (1.0) AB	19.7 (0.9) C	24.1 (1.0) E
WR	51	9.9 (0.9) AB	33.3 (1.8) A	39.1 (1.2) A

BB = Bessent/Byrd Property, BC = Balcones Canyonlands National Wildlife Refuge, CB = Colorado Bend State Park, ER = East Range (Fort Hood), MSER = Eastern Fort Hood, MM = Manning Mountain (Fort Hood), RR = Ridge Road (Fort Hood), TA14 = Training Area 14 (Fort Hood), WF = West Fort Hood, WR = West Range (Fort Hood).

There were significant differences among samples for estimates of A_r, H_e, and H_o ($P < 0.001$ for all comparisions). Site EF had significantly higher A_r than did sites BC and RR (Table 1). Site WR has significantly higher measures of heterozygosity than all other sites; BC had the lowest values (Table 1).

Initial tests of allele frequencies differences indicated that some sites on Fort Hood were not genetically differentiated. Sites on Fort Hood that were not significantly differentiated and in close proximity were combined to produce three overall Fort Hood sites and 6 sites in total. This combining of nearby sites with genetically similar composition was done because treating subsamples of a single population as different populations can bias downstream results. East Range combined (ER_c) included EF and ER individuals, and West Range combined (WR_c) included WR, MM, RR, and TA individuals. The remaining analysis used the resulting 6 sample sites: BB, BC, CB, ER_c, WF, and WR_c. The overall F_{ST} value across our 6 sites was 0.005 ($P < 0.001$). Prior to a

sequential Bonferroni correction, all population pairs between Fort Hood and central Texas sites were significantly differentiated, except between BB and WF. We found nine pairs of populations to be significantly differentiated after a sequential Bonferroni correction (Table 2). Seven of the nine significantly differentiated population pairs were between Fort Hood and central Texas sites. On Fort Hood, only ER$_c$ and WR$_c$ were significantly differentiated. We did not combine Fort Hood sites further as they are separated in space and have a history of being genetically differentiated (Barr *et al.*, 2008; Athrey *et al.*, 2012a).

Table 2. Genetic differentiation between sites sampled for Black-capped Vireos. Pairwise F_{ST} values are depicted on the lower left and P-values are depicted on the upper right. Values that are significant before and after a sequential Bonferroni correction are italicized and bolded, respectively.

	BB	BC	CB	ER$_c$	WF	WR$_c$
BB	-	<0.001	0.005	0.018	0.461	<0.001
BC	**0.013**	-	<0.001	0.024	0.004	0.002
CB	**0.010**	**0.014**	-	0.003	0.023	<0.001
ER$_c$	*0.003*	*0.006*	**0.013**	-	0.521	<0.001
WF	-0.002	**0.009**	*0.011*	0.002	-	0.142
WR$_c$	**0.007**	**0.005**	**0.013**	**0.002**	0.003	-

BB = Bessent/Byrd Property, BC = Balcones Canyonlands National Wildlife Refuge, CB = Colorado Bend State Park, ER = East Range (Fort Hood), WF = West Fort Hood, WR = West Range (Fort Hood).

Seven clusters were identified using the estimated log of probability of the data (lnP(D)) and Deviance Information Criterion (DIC) scores from both STRUCTURE and TESS (-15243.4, 30655.64, respectively). However, summary bar plots from STRUCTURE and TESS show no subdivision and considerable admixture among our study sites (Fig. 1 & 2). We used the 10 lowest DIC value runs to produce the TESS summary plots. A lack of genetic subdivision yet clustering by modeling in these programs may be consistent with limited, but ongoing, gene flow among populations.

K=7

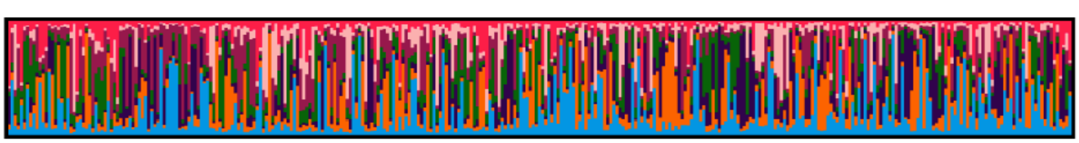

Figure 1. Summary barplot depicting a lack of Black-capped Vireo (*Vireo atricapilla*) genetic structure (STRUCTURE). The colors reflect assignment probabilities to 7 different clusters identified by STRUCT; the lack of geographic pattern suggests spatial structure in gene frequencies does not reflect strongly differentiated populations.

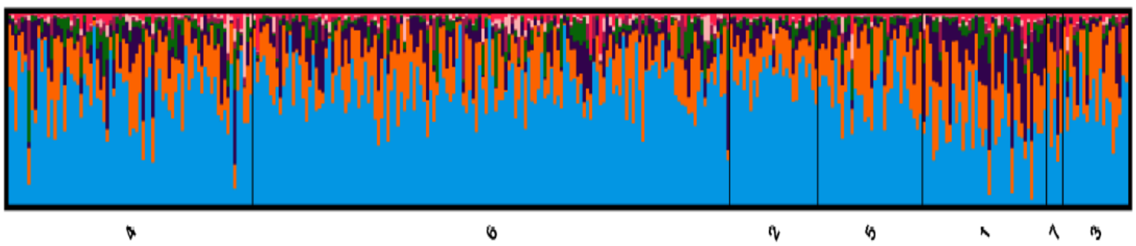

Figure 2. Summary barplot of the ten lowest DIC value runs depicting a lack of Black-capped Vireo (*Vireo atricapilla*) genetic structure (TESS). The colors reflect assignment probabilities to 7 different clusters identified by TESS; the lack of geographic pattern suggests spatial structure in gene frequencies does not reflect strongly differentiated populations.

At both the population and individual levels, we did not observe isolation by distance. At the population level, we found no discernable pattern between log transformed Euclidean distance and linearized F_{ST} when plotted and the IBD test was not statistically significant (r = 0.3001, P = 0.265; Fig. 3). At the individual level, we found no evidence for IBD or spatial autocorrelation.

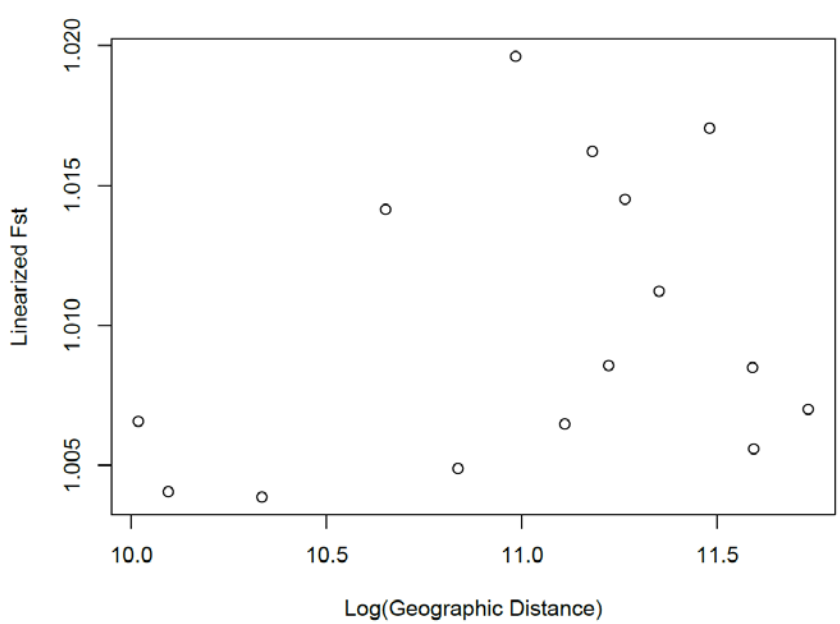

Figure 3. A visualization of linearized Fst plotted against log transformed Euclidean distance, showing a lack of isolation by distance at a population level (R).

Movement

We found consensus that Fort Hood, especially the WR_c site, provides the majority of migrants across our study (BAYESASS, GENECLASS2, MIGRATE-N, CERVUS). Most Black-capped Vireo individuals remained at their putative natal population, based on BAYESASS estimates that ranged from 67.6% (BB and WF_c) to 83.2% (WR_c; Table 3). In general, Fort Hood sites ER_c and WR_c have the highest proportions of individuals that did not disperse. These two sites were also the only sites that had estimates of emigration that were much larger than their SEs (Table 3); most of the other estimates of emigration are probably not different than zero. Only ER_c and WR_c contributed at least 10% of migrants to all other sites, with one exception (from ER_c to BC). All other sites contributed < 5% of migrants with the majority contributing less than 1% of migrants.

MIGRATE-N estimated net emigration from the Fort Hood site WR_c, and net immigration into the rest of the sites (Table 4a). At least 25 migrants from WR_c were estimated in each of the other study sites. When comparing migration within Fort Hood and within central Texas, we found a larger proportion of migration within Fort Hood (average immigration = 60%, average emigration = 52%) than that within Central Texas (average immigration = 18%, average emigration = 24%; Table 4b). However, when looking at movement between central Texas sites and Fort Hood sites, there was an overwhelmingly larger proportion of migrants from Fort Hood to central Texas (average = 82%) than from central Texas to Fort Hood (40%; Table 4c). Overall these migration values indicate considerable movement by Fort Hood individuals, with WR_c providing the most immigrants to other Fort Hood sites and central Texas.

Table 3 Migration rates between populations as the fraction of individuals in population i from population j (BayesAss). Bolded values represent migration rates within a population, e.g. the fraction of individuals that remain in a population. Estimates of migration rates that are twice their standard errors (in parentheses) are italicized.

	pop j					
pop i	BB	BC	CB	ER_c	WF	WR_c
BB	***0.676(0.009)***	0.008(0.008)	0.008(0.008)	*0.187(0.0320)*	0.008(0.008)	*0.114(0.030)*
BC	0.010(0.010)	***0.677(0.010)***	0.010(0.020)	0.042(0.024)	0.010(0.010)	*0.252(0.029)*
CB	0.013(0.013)	0.013(0.013)	***0.680(0.013)***	*0.172(0.0345)*	0.0130(0.012)	*0.110(0.033)*
ER_c	0.010(0.008)	0.006(0.006)	0.0120(0.009)	***0.781(0.020)***	0.005(0.005)	*0.185(0.022)*
WF	0.011(0.010)	0.009(0.009)	0.009(0.009)	*0.148(0.034)*	***0.676(0.009)***	*0.148(0.034)*
WR_c	0.004(0.003)	0.003(0.003)	0.003(0.003)	*0.156(0.030)*	0.003(0.0023)	***0.832(0.030)***

BB = Bessent/Byrd Property, BC = Balcones Canyonlands National Wildlife Refuge, CB = Colorado Bend State Park, ER_c = East Range (Fort Hood), WF_c = West Fort Hood, WR = West Range (Fort Hood).

Table 4 a) Mean number of migrants from pop i to pop j as above (MIGRATE-N). Values in parentheses are the 2.5% and 97.5% values of the posterior distribution, respectively. Bolded values denote estimates with posterior distributions that did not include 0. Below the estimates of the migrants, the table contains summed values of immigration (I) and emigration (E) and net movement (I –E). These quantities were also determined for b) mean number of immigrants and emigrants within central Texas (left) and within Fort Hood (right). c) Mean number of immigrants and emigrants from Fort Hood to central Texas (left) and from central Texas to Fort Hood (right). b) and c) Percentage of total immigration and emigration, and net movement for each study site is tabulated.

a)	pop j					
pop i	BB	BC	CB	ER_c	WF	WR_c
BB	--	**18.7 (1.3-36)**	6.5 (0-23.3)	19.1 (0-37.3)	24.7 (0-44.7)	**23.7 (5.3-41.3)**
BC	8.4 (0-28)	--	3.5 (0-20)	**25.5 (8-42.7)**	**21.9 (4-40)**	**32.4 (14.7-50.7)**
CB	14.1 (0-30)	5.6 (0-26)	--	10.9 (0-26.7)	16.32 (0-32)	17.7 (0-34)
ER	**37.1 (14-61.3)**	**19.4 (2-36)**	13.1 (0-28.7)	--	27.0 (0-63.3)	**55.1 (30-79.3)**
WF	13.2 (0-29.3)	**24.7 (6.7-42)**	11.3 (0-26.7)	15.2 (0-30.7)	--	**25 (6.7-42)**
WR	**49.91 (20-73.3)**	**49.8 (25.3-73.3)**	**33.9 (12-54)**	**118.5 (125.3-171.3)**	**61.8 (42.7-80)**	--
I	122.69	118.35	68.27	189.13	151.76	153.89
E	92.74	91.62	64.59	151.73	89.39	314.02
Net Movement	29.95	26.73	3.68	37.4	62.37	**-160.13**

b)	Within Central Texas			Within Fort Hood		
	BB	BC	CB	ER	WF	WR
I	22.44	24.38	10.06	133.75	88.83	80.13
% of total I	0.18	0.21	0.15	0.71	0.59	0.52
E	25.28	11.86	19.74	82.12	40.21	180.38
% of total E	0.27	0.13	0.31	0.54	0.45	0.57
Net Movement	-2.84	12.52	-9.68	51.63	48.62	-100.25

c)	From Fort Hood to Central Texas			From Central Texas to Fort Hood		
	BB	BC	CB	ER	WF	WR
I	100.25	93.97	58.21	55.38	62.93	73.76
% of total I	0.82	0.79	0.85	0.29	0.41	0.48
E	67.46	79.76	44.85	69.61	49.18	133.64
% of total E	0.73	0.87	0.69	0.46	0.55	0.43
Net Movement	32.79	14.21	13.36	-14.23	13.75	-59.88

BB = Bessent/Byrd Property, BC = Balcones Canyonlands National Wildlife Refuge, CB = Colorado Bend State Park, ER = East Range (Fort Hood), WF = West Fort Hood, WR = West Range (Fort Hood).

We detected 23 migrant individuals (Table 5) with a p-value < 0.01 (GENECLASS2). Most migrants were found on Fort Hood (16; 69.6%), 14 of which were found in WR and ER (60.9% of total migrants). We detected far fewer migrants in the central Texas sites (1-3 migrants), but 13% of detected migrants were found in BB. It is important to note that migrants comprised a larger proportion of estimated population sizes for the central Texas sites (1-4%) compared to the Fort Hood sites (< 0.6%; Table 5).

Table 5 Detected migrants (M), proportion of total migrants detected (Pm), estimated numbers of breeding individuals (N) and proportion of breeding individuals that are migrants (Pn) in each population (GENECLASS2).

Population	M	Pm	N	Pn
BB	3	0.130	78	0.038
BC	1	0.043	88	0.011
BR	1	0.043	26	0.038
CB	2	0.087	136	0.015
ER	6	0.261	1986	0.003
WF	2	0.087	320	0.006
WR	8	0.348	6584	0.001

BB = Bessent/Byrd Property, BC = Balcones Canyonlands National Wildlife Refuge, CB = Colorado Bend State Park, ER = East Range (Fort Hood), WF = West Fort Hood, WR = West Range (Fort Hood).

We used 338 individuals in our parentage analysis in the program CERVUS (Table 6). Maternity and paternity were assigned for 22 and 21 individuals, respectively, of candidate offspring at a 95% confidence interval. At a relaxed confidence level (80%), maternity and paternity were assigned for 50 and 86 individuals for candidate offspring, respectively. Simulations indicated that there would be more paternity assignments than maternity assignments (9%, 9%, respectively at the 95% CI), yet we observed approximately the same percentage. We observed fewer assignments than expected for either confidence interval (Table 6), suggesting that our genotype data had power to detect a greater number of true parent-offspring pairs (assignment power). The odds that an individual was a resident were 10.23 (paternity, $P < 0.0001$) and 7.21 (maternity, $P < 0.0001$). To date, all of the immigrants detected in the central Texas populations were from Ft Hood and no individuals from any of the central Texas populations were found in Ft. Hood. We ae continuing to conduct further additional to further substantiate this conclusion.

Table 6 Summary of parentage assignment analysis (CERVUS). Results of maternity and paternity assignments consisting of observed and expected assignment values and percentages, with associated confidence levels.

Maternity		Assignment		Assignment Percentage	
Level	Confidence (%)	Observed	Expected	Observed	Expected
Strict	95	22	14	9%	6%
Relaxed	80	50	16	21%	7%

Paternity		Assignment		Assignment Percentage	
Level	Confidence (%)	Observed	Expected	Observed	Expected
Strict	95	21	24	9%	10%
Relaxed	80	86	29	36%	12%

Landscape drivers of gene flow and movement

Our results indicate that open habitat, scrub, and human development are likely to play a role in regulating gene flow (Table 7). The models for each of these variables had lower AICc scores than our null model distance. Our models also highlighted a set of optimized parameters for the multiple landscape variables including a value of 10 for open habitat, a value of 0.001 for scrub, 1000 for human development, 0.01 for forest, and 1000 for water and croplands 1000. Forest, water, and croplands had higher AICc scores than our null model distance, indicating that they are unlikely to play a role in gene flow. Elevation and distance were continuous data and as such did not require optimization. All AICc and BIC scores provided consistent estimates of optimized parameters.

With respect to the multivariate models, the best models tended to be those that assigned very low resistance values to shrub or very high values to open habitats reinforcing the role that the two landscape elements likely play in regulating movement and gene flow (Table 8). All multivariate models had AICc values higher than our null model. Notably, all scores for the multivariate models were lower than those for all univariate models, suggesting that multiple landscape features were not interacting to influence gene flow in this system. All AICc and BIC scores were consistent with respect to model rankings.

Table 7. Optimization of landscape variable parameters. Values for the respective variable are beside the variable name. Variables bolded in blue are the optimized parameters for variables with lower AIC, BIC, and AICc scores than our null model. Variables bolded in red are optimized parameters for variables with higher AIC, BIC, and AICc scores than our null model. The null model, emboldened in black, is distance.

Variable	AIC	BIC	AICc	deltaAIC	AICw	BICw
Open 10	**-89.906**	**-87.074**	**-89.787**	**0.000**	**0.105**	**0.105**
Scrub 0.001	**-89.418**	**-86.586**	**-89.300**	**0.487**	**0.082**	**0.082**
Open 100	-89.324	-86.492	-89.206	0.582	0.078	0.078
Scrub 0.01	-89.294	-86.461	-89.175	0.612	0.077	0.077
Open 1000	-89.192	-86.360	-89.074	0.714	0.073	0.073
Scrub 0.1	-88.629	-85.797	-88.511	1.277	0.055	0.055
Elevation	**-88.575**	**-85.743**	**-88.456**	**1.331**	**0.054**	**0.054**
Dev 1000	**-87.996**	**-85.164**	**-87.878**	**1.910**	**0.040**	**0.040**
Dev 100	-87.959	-85.127	-87.841	1.946	0.040	0.040
Scrub 0.5	-87.807	-84.975	-87.689	2.098	0.037	0.037
Distance	**-87.610**	**-84.777**	**-87.491**	**2.296**	**0.033**	**0.033**
Open 2	-87.469	-84.636	-87.350	2.437	0.031	0.031
Dev 10	-87.125	-84.293	-87.007	2.780	0.026	0.026
Forest 0.01	**-87.109**	**-84.277**	**-86.991**	**2.797**	**0.026**	**0.026**
Forest 0.001	-87.062	-84.230	-86.944	2.844	0.025	0.025
Forest 0.1	-86.908	-84.076	-86.789	2.998	0.023	0.023
Forest 0.5	-86.566	-83.734	-86.448	3.340	0.020	0.020
Water 1000	**-86.561**	**-83.729**	**-86.443**	**3.344**	**0.020**	**0.020**
Water 100	-86.530	-83.698	-86.412	3.375	0.019	0.019
Dev 2	-86.508	-83.675	-86.389	3.398	0.019	0.019
Crop 1000	**-86.500**	**-83.668**	**-86.382**	**3.405**	**0.019**	**0.019**
Crop 100	-86.497	-83.665	-86.379	3.408	0.019	0.019
Water 10	-86.486	-83.654	-86.368	3.419	0.019	0.019
Crop 10	-86.484	-83.652	-86.366	3.421	0.019	0.019
Water 2	-86.475	-83.643	-86.357	3.430	0.019	0.019
Crop 2	-86.473	-83.641	-86.355	3.432	0.019	0.019

Table 8. Ranking of models from multivariate linear mixed effects modelling using AIC, BIC, AICc, delta AIC, AIC weights and BIC weights. Models in blue font are those with deltaAIC values lower than 2 and have AIC, BIC, and AICc values lower than the null model. The null model, distance, is bolded.

Model	AIC	BIC	AICc	AICc	deltaAIC	AICw	BICw
Open10	-89.91	-87.07	-89.79	-89.787	0.000	0.275	0.276
Scrub0001	-89.42	-86.59	-89.30	-89.300	0.487	0.216	0.216
Elevation	-88.57	-85.74	-88.46	-88.456	1.331	0.142	0.142
Dev1000	-88.00	-85.16	-87.88	-87.878	1.910	0.106	0.106
Distance	**-87.61**	**-84.78**	**-87.49**	**-87.491**	**2.296**	**0.087**	**0.087**
Forest001	-87.11	-84.28	-86.99	-86.991	2.797	0.068	0.068
Water1000	-86.56	-83.73	-86.44	-86.443	3.344	0.052	0.052
Crop1000	-86.50	-83.67	-86.38	-86.382	3.405	0.050	0.050
Scrub000, Forest001	-81.48	-77.94	-81.30	-81.305	8.482	0.004	0.003
Open10, Dev1000, Crop1000	-64.40	-60.15	-64.15	-64.153	25.635	0.000	0.000
Open10, Scrub0001, Elevation, Dev1000	-57.53	-52.58	-57.20	-57.199	32.589	0.000	0.000
Open10, Scrub0001, Dev1000, Crop1000	-52.35	-47.39	-52.01	-52.013	37.775	0.000	0.000
Open10, Scrub0001, Elevation, Dev1000, Water1000, Distance	-30.03	-23.66	-29.49	-29.494	60.294	0.000	0.000

Conclusions and Implications

Perhaps the most important conclusion to be drawn from these analyses is that the Fort Hood population is likely providing most of the immigrants to the outlying populations. The demographic analysis from RC-2120 indicated that in many years several of these outlying populations like serve as population sinks—because mortality rates tend to be higher than productivity rates. Similarly, those demographic studies indicate that the Fort Hood populations likely serve as sources. The genetic results complete the picture, confirming that birds tend to move from the base to the outlying populations more often than they move in the other direction.

Given that Fort Hood is likely to harbor critical source populations for the region, the management of those populations will likely be critical for the species' persistence in Texas. Previous studies performed under SERDP project RC-1541 and RC-2120 indicated that cowbird control plays a key role in population dynamics and that continued cowbird control is critical for preventing population declines on Fort Hood.

The results of our landscape genetic analyses imply that the loss of scrub habitat in the region may limit movement among population in the region. However, the fact that there is continuous, yet low level, movement—particularly from Fort Hood to the surrounding populations—indicates that the fragmentation of the shrub/scrub habitat has not completely eliminated connectivity among the populations.

As a final note, these results should be considered as preliminary as additional analyses are ongoing.

Literature Cited

Athrey, G., Barr, K. R., Lance, R. F., & Leberg, P. L. 2012a. Birds in space and time: genetic changes accompanying anthropogenic habitat fragmentation in the endangered black-capped vireo (*Vireo atricapilla*). *Evolutionary applications 5*: 540-552.

Barr, K. R., Lindsay, D. L., Athrey, G., Lance, R. F., Hayden, T. J., Tweddale, S. A., & Leberg, P. L. 2008. Population structure in an endangered songbird: maintenance of genetic differentiation despite high vagility and significant population recovery. *Molecular Ecology* 17: 3628-3639.

Fazio III, V. W., Miles, D. B., & White, M. M. 2004. Genetic differentiation in the endangered Black-capped Vireo. *The Condor 106*: 377-385.

Leberg, P. L., G. N. R. Athrey, K. R. Barr, D. Lindsay, and R. Lance. 2009. Implications of landscape alteration for the conservation of genetic diversity of endangered species. In J. A. DeWoody, J. W. Bickham, C. Michler, K. Nichols, O. E. Rhodes, and K. Woeste, eds. *Molecular Insights into Natural Resource Conservation and Management*, pp. 212–238. Cambridge University Press, New York, NY.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13: 55–65.

Raymond, M., & Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of heredity 86*: 248-249.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution 43*: 223-225.

Walker, L. E., Marzluff, J. M., & Cimprich, D. A. 2016. Source-sink population dynamics driven by a brood parasite: A case study of an endangered songbird, the black-capped vireo. *Biological Conservation 203*:108-118.